REMARKS

Upon entry of this amendment, claims 75-100 are pending. Claims 75, 80, 83, 86, 88, 93, 96 and 99 have been amended to more clearly define the present invention. The Examiner has not made any prior art rejections against the pending claims. The amended claims are intended to more clearly define the claimed invention and is not intended to limit the scope of the invention.

Entry of this amendment and consideration of the attached Appendix and the cited prior art is respectfully requested. Applicants submit this data after speaking with the Examiner in April of this year. Applicants did not present this data earlier as the first named inventor died a few years ago. As a result of each assignee changing the main focus of its research, this patent application was licensed to small company that has now taken responsibility of prosecuting this application. Therefore, as a result of the shift in activities and priorities, applicants were not in a position to early present this supportive information. Applicants' again respectfully request consideration of the enclosed information supporting the recited regulatory sequences that they were known to persons skilled in the art. This information is supportive of previously made arguments.

1. Claim Objections

The Examiner objects to a number of claims that he considers to contain typographical errors and to claims in which he wants the article "an" changed to "a." Applicants have amended the claims as requested by the Examiner.

2. Rejections under 35 U.S.C. 112, first paragraph

Claims 75-100 are rejected under 35 U.S.C. 112, first paragraph, as allegedly not being adequately described in the specification. The Examiner states that the claims are enabled for a transgenic non-human mammal, whose genome comprises a transgene comprising a nucleic acid sequence encoding a protein operatively linked to a promoter that causes secretion of the protein into the urine of the transgenic mammal, where the protein is expressed and secreted in the urine of the mammal, and are enabled for a method of producing a protein in the urine of the mammal. However, the Examiner states that the claims are not reasonably enabled for using 5' regulatory sequences of the

present application, scientific publications were available that disclosed promoters from the following genes: uromodulin, uroplakin, renin, erythropoietin, apolipoprotein and aquaporin. Applicants will provide the publications cited in this Appendix by hand-carry to the Examiner by next week. Therefore, in an effort to expedite prosecution, claims 75, 80, 82, 88, 93, and 96 have been amended to recite the 5' and 3' regulatory sequences from these six genes that applicants submit were known and available prior to or at the time that the present application's priority document was filed.

A "patent need not disclose, and preferably omits, what is well known in the art." Hybritech v. Monoclonal Antibodies, Inc., 231 USPQ 81, 94 (Fed. Cir. 1986).

In particular that Examiner has alleged that the specification fails to teach the uromodulin promoter and how it was obtained. In this regard, the Examiner is referred to the publication by Yu *et al.* (1994), that identifies rat, human and bovine promoters. Thus, applicants maintain that the declaration by Dr. Serguei Soukharev, submitted with the previous response, is persuasive in supporting applicants' position that the claims are enabled. These constructs were made using the disclosure of the present invention to produce transgenic mammals that expressed EPO and α1-PI, which is secreted into their urine.

While the specification does not teach "any and all" gene promoters, the regulatory regions of many genes expressed in the urinary tract have been sequenced and are known and available to the skilled artisan. Applicants submit that the skilled artisan is sufficiently skilled to select, isolate, manipulate and prepare constructs containing appropriate 5' and 3' regulatory elements of the described genes expressed in the urinary tract for use in preparing transgenic mammals that express a specific protein or peptide in cells in the urinary tract with the subsequent secretion into the urine. Both independent claims 75 and 88 require that the protein or peptide be expressed under the control of the recited 5' regulatory sequences and then secreted into the urine or detectable in the urine of the mammal, which limits the claims to 5' regulatory sequences that result in the expression of the protein or peptide in the cells of the transgenic mammal with the subsequent secretion of the protein or peptide into the urine of the mammal.

In regard to the 3' regulatory sequences result in the expression of the exogenous gene with subsequent expression into the urine, these sequences are contained in constructs that also contain the recited 5' regulatory sequences of claims 75 and 88 that result in the

expression of the protein or peptide in the cells of the urinary tract of the mammals with the subsequent secretion into the urine of the non-human transgenic mammals. A skilled person is capable of selecting and testing appropriate, functional 3' regulatory sequences without undue experimentation from known genes that encode proteins that are associated with the urinary tract of mammals. In view of the above arguments and Dr. Soukharev's declaration, it is requested that this rejection be withdrawn.

In regard to the Examiner's statement that the specification is not enabled for expressing an enzyme in the urine of transgenic non-human mammals, applicants submit that applicants' Example 1 in the specification discloses expressing Protein C, a protease in the urine. Additionally, Sun (WO 96/9399494) and Sun (U.S. 5,824,543), that were applied as prior art and withdrawn by the Examiner, disclose expressing, β-galactosidase, an enzyme, in the urine under control of the uroplakin promoter. Thus, applicants submit that there is sufficient evidence to support expressing an enzyme in urine. Further, in this regard, the proteins that are expressed in the urine can be assayed to determine if successful expression of the enzyme has occurred. Such protein assays are known to persons skilled in the art and would not require undue experimentation.

In view of the above amendments and arguments, it is requested that this rejection be withdrawn.

3. Rejection under 35 U.S.C. § 112, second paragraph

All of the claims are rejected as allegedly being indefinite for failing to poarticularly point out and distinctly claim the subject matter. The Examiner has not provided any specific basis for this rejection but applicants submit that the claim amendments and arguments provided above are sufficient to overcome the basis of this rejection and it is requested that this rejection be withdrawn.

Conclusion

Entry of this amendment is respectfully requested as it does not raise any new issues and it reduces issues for Appeal. Applicants submit that this application is in condition for allowance, and they solicit an early indication to that effect. Should the Examiner believe that further discussion of any remaining issues would advance the prosecution, a telephone call to the undersigned, at the telephone number listed below, is courteously invited.

Respectfully submitted,

June 30, 2003
Date

Jayme A. Huleatt
Reg. No. 34,485

FOLEY & LARDNER
Suite 500
3000 K Street, NW
Washington, D.C. 20007-5109
(202) 672-5542

MARKED-UP COPY OF CLAIMS

75. (Amended) A method of producing a protein or a peptide in the urine of a non-human transgenic mammal, said method comprising:

(a) providing a non-human transgenic mammal having stably integrated into its genome an exogenous gene encoding a protein or a peptide comprising expression regulatory sequences operably linked to said exogenous gene encoding said protein or peptide; and

(b) allowing said exogenous gene encoding said protein or peptide to be expressed and to be secreted into the urine of said transgenic mammal,

wherein said expression regulatory sequences comprise 5' regulatory sequences selected from the group consisting of [an] **a** uromodulin gene, a renin gene, an erythropoietin gene, an apolipoprotein E gene, [an osteopontin gene, an urinary kallikrein gene, an urinary thrombomodulin gene, an uropontin gene, a nephrocalcin gene] and an aquaporin gene.

80. (Amended) The method of claim 79, wherein said 3' regulatory sequences are selected from the group consisting of [an] **a** uromodulin gene, [an] **a** uroplakin gene, a renin gene, an erythropoietin gene, an apolipoprotein E gene, [an osteopontin gene, an urinary kallikrein gene, an urinary thrombomodulin gene, an uropontin gene, a nephrocalcin gene] and an aquaporin gene.

83. (Amended) The method of claim 82, wherein said 3' regulatory sequences are selected from the group consisting of [an] **a** uromodulin gene, [an] **a** uroplakin gene, a renin gene, an erythropoietin gene, an apolipoprotein E gene, [an osteopontin gene, an urinary kallikrein gene, an urinary thrombomodulin gene, an uropontin gene, a nephrocalcin gene] and an aquaporin gene.

86. (Amended) The method of claim 75, wherein said protein or peptide is selected from the group consisting of prothrombin, Factor VII, Factor IX, Protein C, Protein S, Factor V, Factor VIII, α1-anti-trypsin, antithrombin III, fibrinogen, albumin, an immunoglobulin, a hormone, a growth factor, erythropoietin, a bone morphogenetic protein, and an ion channel protein.

88. (Amended) A non-human transgenic mammal that produces in its urine a protein or peptide, wherein said transgenic mammal has stably integrated into its genome an

exogenous gene encoding a protein or peptide comprising expression regulatory sequences operably linked to said exogenous gene, wherein said expression regulatory sequences comprise 5' regulatory sequences selected from the group consisting of [an] **a** uromodulin gene, a renin gene, an erythropoietin gene, an apolipoprotein E gene, [an osteopontin gene, an urinary kallikrein gene, an urinary thrombomodulin gene, an uropontin gene, a nephrocalcin gene] and an aquaporin gene, and wherein said protein or peptide is detectable in the urine of said transgenic mammal.

93. (Amended) The mammal of claim 92, wherein said 3' regulatory sequences are selected from the group consisting of [an] **a** uromodulin gene, [an] **a** uroplakin gene, a renin gene, an erythropoietin gene, an apolipoprotein E gene, [an osteopontin gene, an urinary kallikrein gene, an urinary thrombomodulin gene, an uropontin gene, a nephrocalcin gene] and an aquaporin gene.

96. (Amended) The mammal of claim 95, wherein said 3' regulatory sequences are selected from the group consisting of a uromodulin gene, a uroplakin gene, a renin gene, a erythropoietin gene, an apolipoprotein E gene, [an osteopontin gene, an urinary kallikrein gene, an urinary thrombomodulin gene, a uropontin gene, a nephrocalcin gene] and a aquaporin gene.

99. (Amended) The mammal of claim 88, wherein said protein or peptide is selected from the group consisting of prothrombin, Factor VII, Factor IX, Protein C, Protein S, Factor V, Factor VIII, α1-anti-trypsin, antithrombin III, fibrinogen, albumin, an immunoglobulin, a hormone, a growth factor, erythropoietin, a bone morphogenetic protein, and an ion channel protein.

APPENDIX A

Number	Promoters	Species	Date	Reference
1.	Uromodulin	Human uromodulin identified as Tamm-Horsfall Protein	1987	Pennica; spec pg 6 [0047]
2	Uromodulin	MRNA of Uromodulin localized to Henles loop	1990	
	Uromodulin	Gene located to chromosome 13	1993	Pook et al.
3	Uromodulin	Rat, human and bovine genes cloned & promoters identified	1994	Yu et al.
4	Uromodulin	Assigned to chromosomes in mouse & rat	1997	Fukuoka et al
5	Uromodulin	Goat promoter identified & sequenced	*1999*	Karatzas C, Personal communication, unpublished
6	Uromodulin	Mouse promoter identified	*2002*	Zhu et al.
7	Uromodulin	Human promoter used to generate transgenic mice expressing human EPO & A1AT	*2002*	Zbikowska
9	Uromodulin	Bovine promoter identified & sequenced; used to generate lac Z transgenic mice	*2003*	Kim et al.
	Uromodulin	Mouse promoter used to generate transgenic mice expressing human growth hormone.	*2003*	Zhu et al.
	Uroplakin	Identification of UPA cDNA	1994	Lin et al., spec pg 7 [0049]
	Uroplakin	Use of UPA promoter to express lac Z in transgenic mice	1995	Lin et al., spec pg 7 [0049]
	Renin	Use of renin promoter & gene in transgenic mice	1988	Mullins et al
	Renin	Tissue-specific expression of lac Z in mice	1990	Sigmund et al.
	Renin	Tissue-specific transactivation in transgenic mice	1994	Fukamizu
	Renin	New function of elements identified in renin promoter	*2001*	Germain 2001
	Erythropoietin	Transgenic mice created using EPO sequences	1991, 1994	Semenza
	Erythropoietin	EPO promoter was used to drive lac Z gene expression	Apr 1997	Haider et al.
	Apolipoprotein E	Human, mouse and rat genes and promoters known	1990	Simonet et al.; Spec pg 8, [0060, 0061]
	Aquaporin	AQP CD isolated	Sept. 1994	Uchida et al., spec pg 7 [0054]
	Aquaporin	AQP-3 isolated	July 1995	Inase et al.
	Aquaporin	AQP2 promoter isolated	June 1996	Hozawa et al
	Aquaporin	Human, rat and mouse AQP2	August 1997	Rai et al.
	Aquaporin	Human AQP-1 promoter isolated	*Feb 1998*	Umenishi et al
	Aquaporin	AQP-4 alternative promoters identified	*June 1998*	Umenishi et al
	Aquaporin	AQP2 promoter used to target the collecting tubules in transgenic mice	*1999*	Stricklett et al

References Cited:

Inase N, Fushimi K, Ishibashi K, Uchida S, Ichioka M, Sasaki S, Marumo F. J Biol Chem. 1995 Jul 28;270(30):17913-6. Isolation of human aquaporin 3 gene.

Uchida S, Sasaki S, Fushimi K, Marumo F. J Biol Chem. 1994 Sep 23;269(38):23451-5 Isolation of human aquaporin-CD gene.

Rai T, Uchida S, Marumo F, Sasaki S.: Am J Physiol. 1997 Aug;273(2 Pt 2):F264-73. Cloning of rat and mouse aquaporin-2 gene promoters and identification of a negative cis-regulatory element.

Simonet WS, Bucay N, Lauer SJ, Wirak DO, Stevens ME, Weisgraber KH, Pitas RE, Taylor JM. In the absence of a downstream element, the apolipoprotein E gene is expressed at high levels in kidneys of transgenic mice. J Biol Chem. 1990 Jul 5;265(19):10809-12.

Mullins JJ, Sigmund CD, Kane-Haas C, Wu CZ, Pacholec F, Zeng Q, Gross KW. Studies on the regulation of renin genes using transgenic mice. Clin Exp Hypertens A. 1988;10(6):1157-67

Sigmund CD, Jones CA, Fabian JR, Mullins JJ, Gross KW. Tissue and cell specific expression of a renin promoter-reporter gene construct in transgenic mice. Biochem Biophys Res Commun. 1990 Jul 16;170(1):344-50.

Fukamizu A, Sagara M, Sugiyama F, Yagami K, Murakami K. Tissue-specific trans-activation of renin gene by targeted expression of adenovirus E1A in transgenic mice. Biochem Biophys Res Commun. 1994 Feb 28;199(1):183-90

Ding Y, Davisson RL, Hardy DO, Zhu LJ, Merrill DC, Catterall JF, Sigmund CD. The kidney androgen-regulated protein promoter confers renal proximal tubule cell-specific and highly androgen-responsive expression on the human angiotensinogen gene in transgenic mice. J Biol Chem. 1997 Oct 31;272(44):28142-8.

Semenza GL, Koury ST, Nejfelt MK, Gearhart JD, Antonarakis SE. Cell-type-specific and hypoxia-inducible expression of the human erythropoietin gene in transgenic mice. Proc Natl Acad Sci U S A. 1991 Oct 1;88(19):8725-9.

Semenza GL. Erythropoietin gene expression in transgenic mice and human hepatoma cells. Ann N Y Acad Sci. 1994 Apr 15;718:41-7; discussion 47-9.

Haidar MA, Loya F, Yang Y, Lin H, Glassman A, Goldwasser E, Albitar M. Electron microscopic localization of lacZ expression in the proximal convoluted tubular cells of the kidney in transgenic mice carrying chimeric erythropoietin/lacZ gene constructs. J Struct Biol. 1997 Apr;118(3):220-5.

Zhu X, Cheng J, Gao J, Lepor H, Zhang ZT, Pak J, Wu XR. : Am J Physiol Renal Physiol. 2002 Apr;282(4):F608-17. Isolation of mouse THP gene promoter and demonstration of its kidney-specific activity in transgenic mice.

Yu H, Papa F, Sukhatme VP. Gene Expr. 1994;4(1-2):63-75. Bovine and rodent tamm-horsfall protein (THP) genes: cloning, structural analysis, and promoter identification.

Pook MA, Jeremiah S, Scheinman SJ, Povey S, Thakker RV. Ann Hum Genet. 1993 Oct;57 (Pt 4):285-90. Localization of the Tamm-Horsfall glycoprotein (uromodulin) gene to chromosome 16p12.3-16p13.11.

Pennica D, Kohr WJ, Kuang WJ, Glaister D, Aggarwal BB, Chen EY, Goeddel DV. Science. 1987 Apr 3;236(4797):83-8 Identification of human uromodulin as the Tamm-Horsfall urinary glycoprotein.

Fukuoka S, Matsuda Y. Cytogenet Cell Genet. 1997;79(3-4):241-2. Assignment of the Tamm-Horsfall protein/uromodulin gene (Umod) to mouse chromosome bands 7F1-F2 and rat chromosome bands 1q36-->q37 by in situ hybridization.

Publications dated post-1997:

Umenishi f & Verkman AS. Genomics 1998 47(3): 341-9. Isolation of the human aquaporin-1 promoter and functional characterization in human erythroleukaemia cell lines.

Stricklett et al. Exp Nephrol 1999, 7 (1): 67-74. Targeting collecting tubules using the aquaporin-2 promoter.

Zbikowska HM, Soukhareva N, Behnam R, Chang R, Drews R, Lubon H, Hammond D, Soukharev S.: Transgenic Res. 2002 Aug;11(4):425-35. The use of the uromodulin promoter to target production of recombinant proteins into urine of transgenic

animals.

Zbikowska HM, Soukhareva N, Behnam R, Lubon H, Hammond D, Soukharev S. Uromodulin promoter directs high-level expression of biologically active human alpha1-antitrypsin into mouse urine. Biochem J. 2002 Jul 1;365(Pt 1):7-11.

Kim HT, Song IY, Piedrahita J.: Transgenic Res. 2003 Apr;12(2):191-201. Kidney-specific activity of the bovine uromodulin promoter.

Zhu X, Cheng J, Huang L, Gao J, Zhang ZT, Pak J, Wu XR.: Transgenic Res. 2003 Apr;12(2):155-62. Renal tubule-specific expression and urinary secretion of human hormone: a kidney-based transgenic bioreactor growth.

Jiang XJ, Feng T, Chang LS, Kong XT, Wang G, Zhang ZW, Guo YL. Urol Res. 1998;26(6):389-94. Expression of osteopontin mRNA in normal and stone-forming rat kidney.

Shiraga H, Min W, VanDusen WJ, Clayman MD, Miner D, Terrell CH, Sherbotie JR, Foreman JW, Przysiecki C, Neilson EG, et al. Proc Natl Acad Sci U S A. 1992 Jan 1;89(1):426-30 Inhibition of calcium oxalate crystal growth in vitro by uropontin: another member of the aspartic acid-rich protein superfamily.